# Defective FXR-FGF15 signaling and bile acid homeostasis in cystic fibrosis mice can be restored by the laxative polyethylene glycol

Bertolini, Anna; van de Peppel, Ivo P.; Doktorova-Demmin, Marcela; Bodewes, Frank A. J. A.; de Jonge, Hugo; Bijvelds, Marcel; Verkade, Henkjan J.; Jonker, Johan W.

*Published in:*
American Journal of Physiology. Gastrointestinal and Liver Physiology

*DOI:*
10.1152/ajpgi.00188.2018



*Publication date:*
2019

# Defective FXR-FGF15 signaling and bile acid homeostasis in cystic fibrosis mice can be restored by the laxative polyethylene glycol

**Running title**: Laxative restores FXR-FGF15 signaling in CF mice

**Authors:**

1. Anna Bertolini[1,2]
2. Ivo P. van de Peppel[1,2]
3. Marcela Doktorova-Demmin[1]
4. Frank A. J. A. Bodewes[2]
5. Hugo de Jonge[3]
6. Marcel Bijvelds[3]
7. Henkjan J. Verkade[1,2]
8. Johan W. Jonker[1,4]

[1] Section of Molecular Metabolism and Nutrition, Laboratory of Pediatrics, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands.

[2] Pediatric Gastroenterology and Hepatology, University of Groningen, University Medical Center, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands.

[3] Gastroenterology & Hepatology, Erasmus MC-University Medical Center Rotterdam, The Netherlands.

[4] To whom correspondence should be addressed.

**Declarations of interest**: none.

**Author contributions**: AB, IvdP and MD performed experiments, analyzed and interpreted data. HJV, JWJ, FAJAB, MD and IPvdP designed the experiments. HJV, JWJ, FAJAB, HdJ and MB supervised research and interpreted data. AB, IPvdP, HJV and JWJ wrote the manuscript.

**Corresponding author**:

Prof. dr. J. W. Jonker

Section of Molecular Metabolism and Nutrition, Laboratory of Pediatrics, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands.

Telephone: +31-503611261

Email: j.w.jonker@umcg.nl

## ABSTRACT

The gastrointestinal phenotype of cystic fibrosis (CF) features intestinal bile acid (BA) malabsorption, impaired intestinal farnesoid X receptor (FXR) activation and consequently reduced fibroblast growth factor 19 (FGF19, FGF15 in mice) production. The osmotic laxative polyethylene glycol (PEG) has been shown to decrease intestinal mucus accumulation in CF mice and could, by doing so, improve BA reabsorption. Here we determined the effect of PEG on BA excretion and FXR-FGF15 signaling in CF mice. Male *Cftr*$^{-/-tm1Unc}$ (CF) and wild type (WT) littermates were administered PEG 4000 in drinking water and fed either chow or a semisynthetic diet. PEG was withdrawn for three days before termination. Fecal BA excretion was measured at PEG dosages of 37 g/L (100%) and 0 g/L (0%). Ileal FXR activation was assessed by gene expression of its downstream targets *Fgf15* and *Shp*. In CF mice, PEG withdrawal increased fecal BA excretion on either diet as compared to full PEG dosage (chow, 2-fold, $p$=0.06; semisynthetic, 4.4-fold, $p$=0.007). PEG withdrawal did not affect fecal BA excretion in WT mice on either diet. After PEG withdrawal, gene expression levels of intestinal FXR target genes *Fgf15* and *Shp* were decreased in CF mice, but unaffected in WT littermates. PEG did not affect the gene expression of the main intestinal BA transporter ASBT. PEG treatment ameliorates intestinal BA malabsorption in CF mice and restores intestinal FXR-FGF15 signaling, independently from *Asbt* gene expression. These findings highlight the potential of PEG in the prevention and treatment of the gastrointestinal phenotype of CF.

**New & Noteworthy**: A gastrointestinal feature of cystic fibrosis is bile acid malabsorption and consequent impairment of FXR-FGF15 signaling. FXR-FGF15 signaling regulates various metabolic processes and could be implicated in metabolic and gastrointestinal complications of cystic fibrosis, such as diabetes and liver disease. In cystic fibrosis mice,

treatment with the osmotic laxative polyethylene glycol is associated with decreased fecal bile acid loss and restoration of FXR-FGF15 signaling.

**Keywords**: cystic fibrosis, bile acids, FXR, FGF15, polyethylene glycol

## INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the *CFTR* gene. CFTR functions as an ion channel to regulate chloride and bicarbonate transport and water volume on epithelial surfaces (25). In CF, reduced CFTR function in the epithelia of mucin-producing organs leads to the accumulation of viscous mucus, which promotes obstruction, infection and inflammation (12). Although the main cause of death in CF is lung disease (25), metabolic and gastrointestinal manifestations are becoming more frequent due to increased life expectancy thanks to improved treatment of pulmonary complications. The most prominent metabolic complication is CF-related diabetes mellitus (CFRD), affecting one third of patients (16). The CF gastrointestinal phenotype is characterized by obstruction, microbial dysbiosis and inflammation (21). Gastrointestinal complications include meconium ileus in the first days of life, as well as malnutrition in infancy. Exocrine pancreatic insufficiency and various degrees of CF-related liver disease (CFLD) mostly ensue during childhood. As patients age, abdominal pain, constipation and the more severe distal intestinal obstruction syndrome (DIOS) further decrease their quality of life (25). Impairment of gut health affects numerous processes in the body (34). In CF, intestinal dysbiosis and subsequent chronic low-grade inflammation are linked to gastrointestinal malignancies, CFLD, CFRD, osteoporosis, and increased cardiovascular risk (19). Improving gut health in CF may thus improve several complications of this multiorgan disease.

The gastrointestinal phenotype of CF is further characterized by increased fecal loss of bile acids (BA) in both patients (24) and CF mouse models (3, 4, 6, 11, 36). BAs are synthesized by the liver and secreted into the duodenum, where they aid in fat absorption. Under physiological conditions, ~95% of secreted BAs are reabsorbed by the small intestine, mostly via the apical sodium-dependent bile acid transporter (ASBT, SLC10A2), to be returned to the liver and thereby complete the enterohepatic circulation (18). In CF,

intestinal reabsorption of BAs is impaired, resulting in increased fecal BA loss (3, 4, 6, 11, 24, 36). Besides their role in fat absorption, BAs exert important metabolic effects, mainly via the BA-sensing farnesoid X receptor (FXR) and its target fibroblast growth factor 19 (FGF19 in humans, FGF15 in mice) (18). Upon reabsorption, BAs activate FXR in ileal enterocytes, resulting in FGF15/19 production. FGF19 travels to the liver via portal blood to exert negative feedback on BA synthesis (18). In CF, BA malabsorption and possibly other mechanisms result in defective FXR-FGF19 signaling, as suggested by reduced ileal *Fgf15* mRNA levels in mice (8) and reduced serum FGF19 in patients (28). In patients, reduced FGF15/19 levels are associated with high fasting plasma glucose and type 2 diabetes (10). In lean mice, Fgf15 deficiency resulted in glucose intolerance and diminished hepatic glycogen storage (17). Additionally, FGF19 administration protects against sclerosing cholangitis (38) and steatosis (39), lesions similar to those observed in CFLD. Impaired FXR-FGF19 signaling may therefore be implicated in the development and/or progression of CF complications such as CFLD and CFRD. Thus, restoring BA homeostasis in CF is an attractive avenue to improve CF complications.

The mechanism underlying BA malabsorption in CF is unclear, however two hypotheses prevail. Firstly, the thickened intestinal mucus layer could impair the translocation of BAs from the lumen to the epithelium for their reabsorption. Secondly, intestinal dysbiosis could promote bacterial BA deconjugation and thereby decrease BA reabsorption, as ASBT preferentially transports conjugated rather than deconjugated BAs (13). Moreover, CF-mediated changes in ASBT expression or functionality could be involved. Some of the factors mentioned in these hypotheses were improved in CF mice upon treatment with the osmotic laxative polyethylene glycol (PEG) (22). PEG is routinely administered to mice lacking *Cftr* expression to prevent development of lethal intestinal obstruction (7). PEG decreased mucus accumulation in the small intestine, intestinal bacterial load, and the expression of certain inflammatory genes (22). We therefore

hypothesized that PEG treatment could improve the reabsorption of BAs in CF. In this study, we aimed to determine the effect of PEG treatment on BA malabsorption and FXR signaling in CF mice. Our results indicate that indeed PEG treatment is associated with decreased fecal BA loss, as well as increased FXR-FGF15 signaling.

## METHODS

*Animals*

Male *Cftr*$^{-/-}$ (*Cftr*$^{tm1UNC}$ on a >99% C57BL/6 background, CF) mice (n=15) and wild-type (WT) littermates (n=15) aged 8-20 weeks obtained from an in-house breeding colony were housed individually under conventional (non-specific pathogen-free) housing conditions in a light- and temperature-controlled facility (12-hour light-dark cycles, 21°C) with *ad libitum* access to water and food. Two diets were used to account for outcome dependency on dietary factors. The mice received either chow [RM3 (E) FG, Special Diet Services, England; composition by proximate analysis: fat 4.3% (cholesterol 0.05%), protein 22.4%, fiber 4.2% (of which 25% cellulose, 57% hemicellulose, 9% pectin, and 9% lignin), nitrogen-free extract 51.2%), or a semisynthetic diet (No. 4063.02, AB diets, The Netherlands; composition: fat 5.2% (cholesterol 0.01%), protein 17.3%, fiber (100% cellulose) 10.5%, nitrogen-free extract 55.7%]. Animal experiments were approved by the Ethics Committee for Animal Experiments of the University of Groningen. All experiments were performed in accordance with relevant guidelines and regulations (including laboratory and biosafety regulations).

*Experimental procedures*

PEG (polyethylene glycol 4000 with electrolytes, Ipsen Farmaceutica, The Netherlands, containing, in g/l: 32 PEG 4000, 0.73 NaCl, 0.375 KCl, 0.84 NaHCO3, and 2.85 Na2SO4, tot. 37g/l) was administered via drinking water in decreasing concentrations. All mice, irrespective of their genotype, were administered PEG (37 g/l water) since weaning to prevent the intestinal obstruction often observed in these CF mice (7). On day 0, PEG dosage was decreased by 50% (18.5 g/l water) to determine the PEG-dependency of CF mice. On day 7, PEG treatment was stopped for three days until termination. Fecal pellets were collected over a 24-hour period before decreasing PEG dosage (day 0, 100% PEG)

and daily from day 8 to 10 (0% PEG). This procedure was followed for both groups, the one receiving chow (CF n=5, WT n=4) and the other receiving semisynthetic diet (CF n=3, WT n=5). Additionally, a separate group of mice (CF n=7, WT n=6) fed semisynthetic diet was administered PEG at full dosage (37 g/L water) until termination and was included for ileal gene expression only. Mice were anesthesized with isoflurane and euthanized by cervical dislocation. Terminal blood samples were collected in EDTA-coated tubes. Tissues were collected and immediately frozen in liquid nitrogen.

*Analytical methods*

*Neutral sterol (NS) and bile acid (BA) analyses*. NS and BAs were extracted and measured by gas chromatography (GC) as previously described (32). Total amounts were calculated as the sum of the individual species. BA species included: α-muricholic acid, β-muricholic acid, chenodeoxycholic acid, cholic acid, deoxycholic acid, hyodeoxycholic acid, ω-muricholic acid and ursodeoxycholic acid. NS species included: cholesterol, coprostanol and dihydrocholesterol.

*Gene expression analysis*. The small intestine was divided into three segments of equal length. Total RNA was isolated from mid-sections of the most distal of the three segments (ileum) with TRI-Reagent (Sigma, St. Louis, MO, USA) and quantified by NanoDrop (NanoDrop Technologies, Wilmington, DE, USA). Primers were designed using Primer-BLAST and optimized for use with Hi-ROX SensiMix™ SYBR Green master mix (Bioline, Taunton, MA, USA). Primers used are listed in **Table 1**. Real-time qPCR analyses were performed on a StepOnePlus™ Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Gene expression levels were normalized to 36B4 (*Rplp0*).

| Gene | Forward primer 5'---3' | Reverse primer 3'---5' |
|---|---|---|
| *Fgf15* | GCC ATC AAG GAC GTC AGC A | CTT CCT CCG AGT AGC GAA TCA G |
| *Shp* | AAG GGC ACG ATC CTC TTC AA | CTG TTG CAG GTG TGC GAT GT |

| | | |
|---|---|---|
| *Asbt* | ACC ACT TGC TCC ACA CTG CTT | CCC GAG TCA ACC CAC ATC TT |
| *Gata4* | GAG ATG CGC CCC ATC AAG | GAC ACA GTA CTG AAT GTC TGG GAC AT |
| *Rplp0* | CTG TTG GCC AAT AAG GTG CC | GGA GGT CTT CTC GGG TCC TA |

**Table 1** - qPCR primer sequences used in this study.

*Statistical analyses.* GraphPad Prism v6.0 for Macintosh (GraphPad Software, La Jolla, CA, USA) was used for data analyses. We analyzed data using a mixed-model ANOVA with genotype as between-subjects factor, and PEG treatment as within-subjects factor using SPSS v25.0 for Windows IBM SPSS Statistics for Windows, Version 25.0 (IBM, Armonk, NY). Statistical differences were subsequently tested using the Student's T-test for unpaired data and the paired T-test for paired data. For correlation analyses, Spearman's rank correlation coefficient was used. Alpha was set at 0.05. In figures 1-4, data concerning 100% PEG dosage refers to 24-hour feces collected on day 0. Data concerning 0% PEG dosage represents the average of 24-hour feces collected on days 8, 9 and 10.

## RESULTS

### PEG treatment ameliorates bile acid malabsorption in CF mice

To investigate the effect of PEG on BA malabsorption in CF mice, PEG was reduced stepwise until complete withdrawal. All mice survived without signs of bowel obstruction or overt diarrhea. The body weight of CF mice tended to be lower than that of WT, however statistical significance was not reached (data not shown). The fecal output was higher in mice fed chow compared to mice fed the semisynthetic diet (**Fig. 1A vs. 1B**), despite similar food intake (data not shown). PEG withdrawal decreased the fecal output in WT mice on either diet (**Fig. 1A,B**), but not in CF mice.

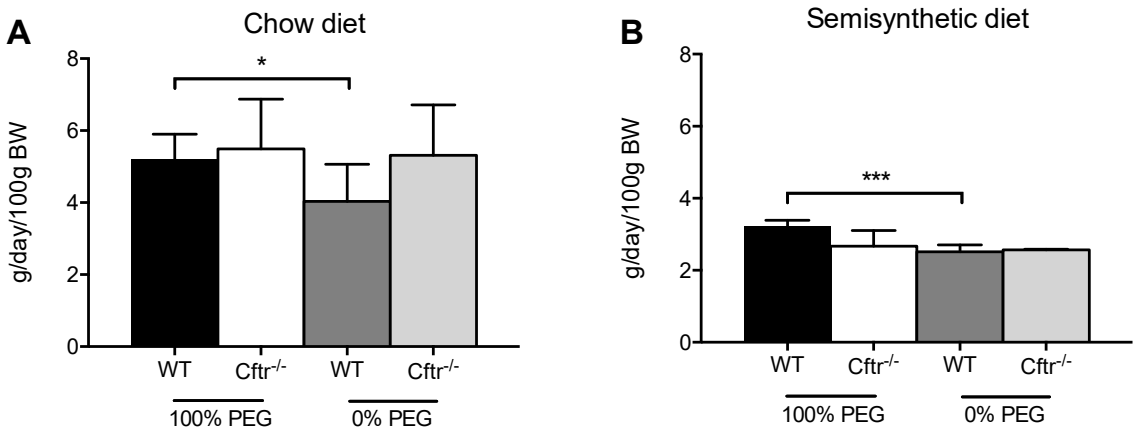


**Figure 1.** Effect of PEG on fecal output in WT and CF mice maintained on (A) chow and (B) semisynthetic diet. Data refers to dry fecal weight and was normalized to body weight. Data are presented as mean±SD, n=3-5. Data of WT mice was compared with that of CF mice by Student's T test. Within-individual mouse changes in fecal output with 100% or 0% PEG treatments were compared by paired T test. PEG: polyethylene glycol.

PEG withdrawal increased fecal BA excretion by two-fold in CF mice receiving a chow diet (**Fig. 2A**). In contrast, PEG withdrawal exerted little effect on the fecal BA excretion in WT mice (**Fig. 2A**).

In CF mice, there is high variability in the absolute amount of fecal BAs observed in previous studies (3, 4, 6, 11, 36), which might be related to the diet, genetic background or environmental factors. In a previous study, fecal BA excretion was lower in rats fed a semisynthetic diet compared to chow (14). To investigate dependency of the outcome on diet, we also performed the same experiment with a semisynthetic diet, which has a different fiber content and composition. Compared to the groups maintained on chow, mice receiving semisynthetic diet showed a 5-to-10-fold lower fecal excretion of BAs (**Fig. 2A vs. 2B**). With PEG, fecal BA excretion was similar between CF and WT mice on a semisynthetic diet (**Fig. 2B**), whereas in those fed chow this was different between the genotypes (**Fig. 2A**). In CF mice fed a semisynthetic diet, PEG withdrawal increased fecal BA excretion by about 4-fold (**Fig. 2B**). As observed on chow, PEG did not affect fecal BA excretion in WT mice (**Fig. 2B**). These findings indicate that PEG improves BA malabsorption in CF mice, on either diet.

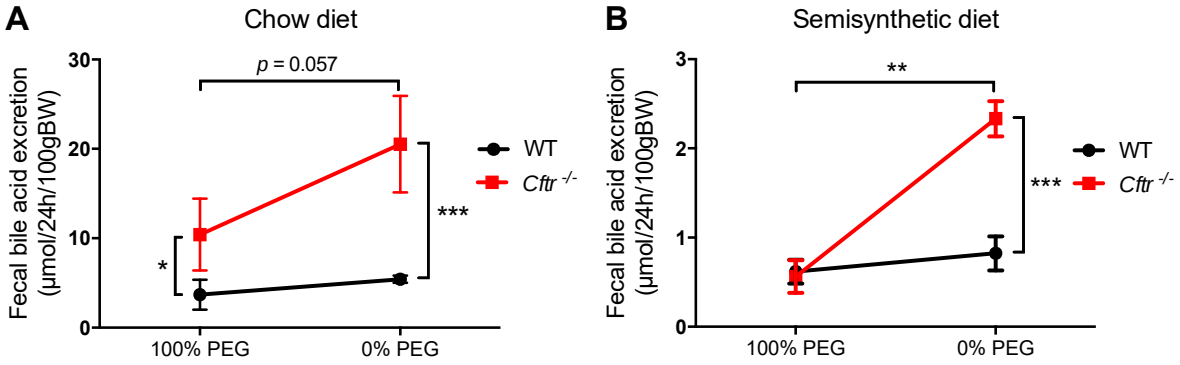


**Figure 2.** Effect of PEG on fecal BA excretion in WT and CF mice maintained on (A) chow and (B) semisynthetic diet. Fecal BA excretion was determined by gas chromatography and normalized to body weight. Data are presented as mean±SD, n=3-5. Data of WT mice was compared with that of CF mice by Student's T test. Potential changes in fecal BA excretion in individual animals, as a result of PEG withdrawal, were assessed by a paired T test.

## PEG treatment does not affect fecal neutral sterol excretion

Since BAs are essential for intestinal absorption of fat, including cholesterol, fecal neutral sterol (NS) excretion was determined (**Fig. 3**). This was lower in mice receiving semisynthetic diet as compared to chow (**Fig. 3A vs. 3B**). In WT mice on either diet, PEG withdrawal was associated with a decrease in fecal NS excretion (**Fig. 3A,B**). Fecal NS excretion was higher in CF as compared to WT mice fed chow, independent of PEG treatment (**Fig. 3A**). Upon semisynthetic diet, fecal NS excretion was similar between CF and WT mice and was unaffected by PEG in CF mice (**Fig. 3B**). We found a positive relationship between fecal BA and NS excretion (**Fig. 3C**). Interestingly, coprostanol, a cholesterol metabolite formed by intestinal microbial conversion, was only found in 1 out of 8 mice fed a semisynthetic diet, whereas it was found in all mice of either genotype fed chow (data not shown).

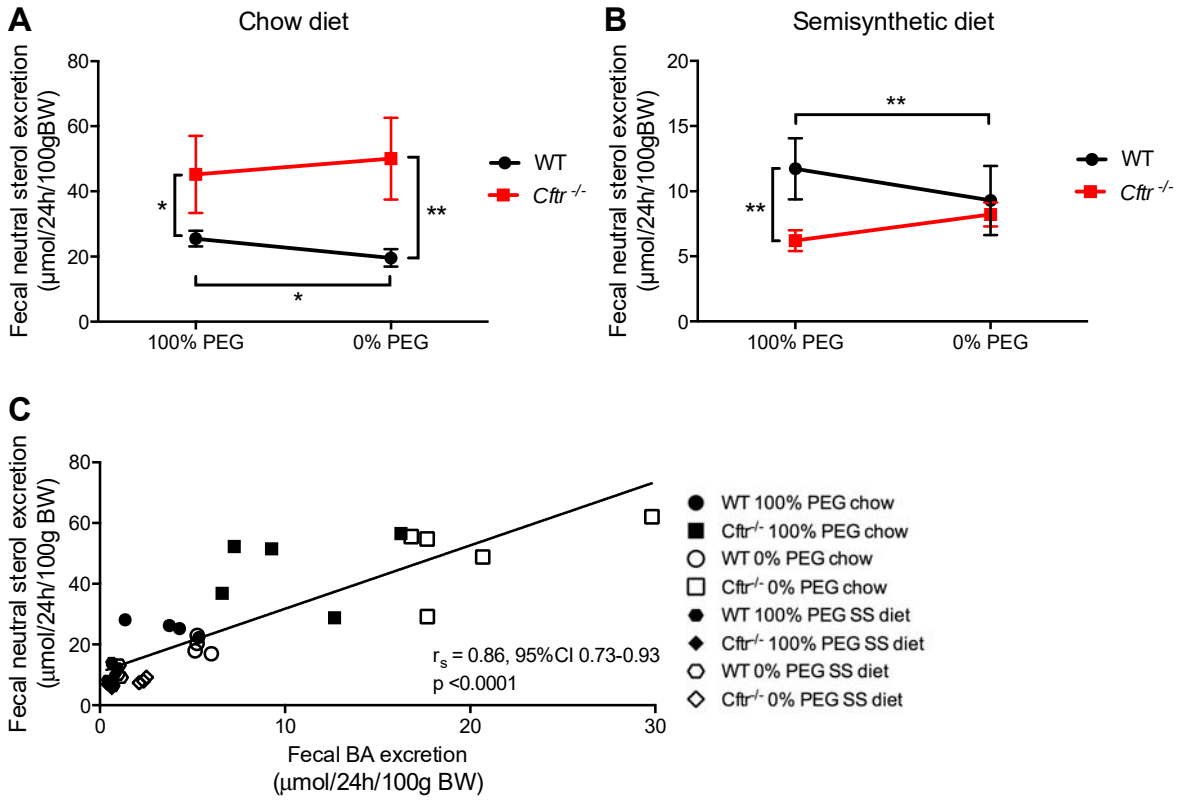

**Figure 3.** Effect of PEG and diet on fecal neutral sterol (NS) excretion in WT and CF mice maintained on (A) chow and (B) semisynthetic diet. Fecal NS excretion was determined by gas chromatography and normalized to body weight. Data is presented as mean±SD, n=3-5. Data of WT mice was compared with that of CF mice by Student's T test. Within-individual mouse changes in fecal NS excretion while receiving 100% or 0% PEG treatment were compared by paired T test. (C) Correlation plot between fecal NS excretion and fecal BA excretion, including data from Fig. 2A,B and Fig. 3A,B. For correlation analyses, Spearman's rank correlation coefficient was used. PEG, polyethylene glycol.

**PEG treatment partly normalizes the fecal BA composition in CF mice**

The fecal BA composition is altered in CF patients and mice, in whom the contribution of the primary BA cholic acid (CA) is high and that of deoxycholate (DCA) is generally low (4, 33, 36). We also found that the contribution of CA to the fecal BA composition was substantially higher in untreated CF as compared to WT mice (**Fig. 4**), and this difference in CA contribution among the two genotypes was reduced by PEG treatment (**Fig. 4**). PEG treatment decreased the CA contribution in CF mice (**Fig. 4**). The contribution of the primary BA chenodeoxycholic acid (CDCA), a potent FXR activator, to the fecal BA composition, tended to be lower in untreated CF as compared to WT mice, and tended to be increased by PEG treatment in CF mice (**Fig. 4**). The contribution of β-muricholic acid (β-MCA) to the fecal BA composition was decreased in untreated CF as compared to WT mice, and was increased by PEG in CF mice (**Fig. 4**). Together, these findings indicate that PEG partially restored imbalances in the fecal BA composition in CF mice. In contrast with previous studies in CF and WT mice fed a liquid diet (4, 36), no fecal deoxycholic acid (DCA) was detected.

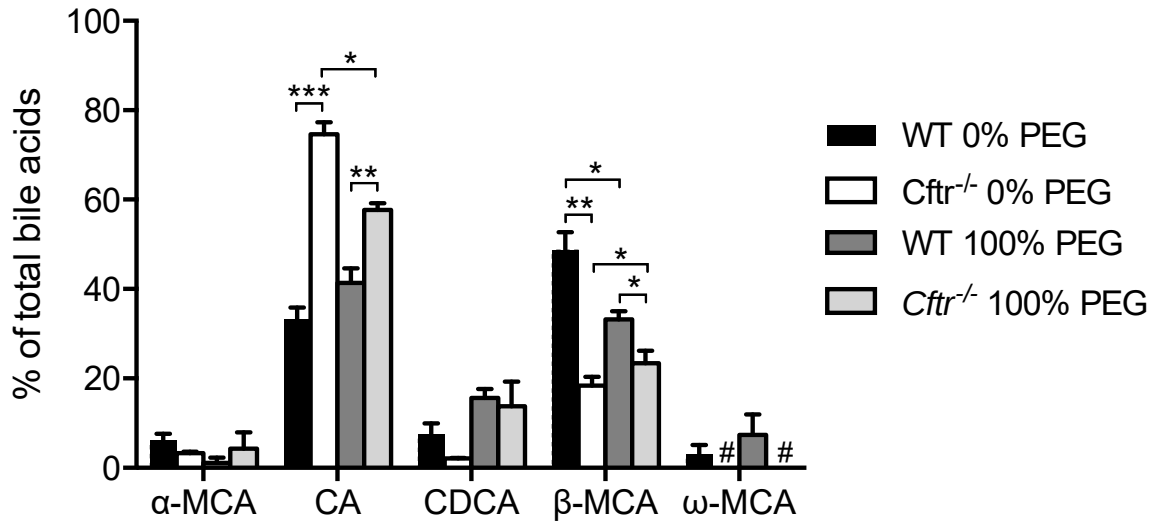


**Figure 4.** Effect of PEG on the fecal BA composition in mice fed semisynthetic diet. Data is shown as percentages of total fecal bile acids. Individual BA species were detected by gas chromatography. Bile acid species include: α-MCA, α-muricholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; β-MCA, β-muricholic acid; ω-MCA, ω-muricholic acid. n=3-5. Data of WT mice was compared with that of CF mice by Student's T test. Within-individual mouse changes in fecal BA composition while receiving 100% or 0% PEG treatment were compared by paired T test. PEG, polyethylene glycol.

**PEG treatment restores FXR-FGF15 signaling in CF mice**

To investigate the effect of decreased fecal BA excretion on FXR signaling, we measured ileal gene expression levels of its downstream targets, *Fgf15* and small heterodimer partner (*Shp, NR0B2)* in the ileum, where BA reabsorption is most pronounced. With PEG treatment, *Fgf15* and *Shp* mRNA levels were similar between CF and WT mice fed a semisynthetic diet (**Fig. 5A**). In contrast, after PEG withdrawal, both *Fgf15* and *Shp* expression were suppressed in CF compared to WT mice. This suppression was stronger in mice receiving chow (**Fig. 5B,C**). In WT mice, PEG treatment did not affect Fgf15 or Shp gene expression. We found a strong inverse correlation

between fecal BA excretion and *Fgf15* expression and between fecal BA excretion and *Shp* expression, indicating that increased fecal BA excretion was associated with lower gene expression of the FXR target genes *Fgf15* and *Shp* (**Fig. 5D,E**). No correlation was observed between CDCA levels and Fgf15 gene expression (data not shown). Interestingly, PEG had no major effect on the expression of the main intestinal BA transporter, *Asbt*. However, without PEG treatment, its expression tended to be lower in CF mice fed semisynthetic diet as compared to WT mice (**Fig. 5A,C**). The transcription factor *Gata4*, known to repress expression of *Asbt* (27), was unchanged in CF as compared to WT mice on both diets (**Fig. 5A-C**). Accordingly, we found no correlation between *Asbt* and *Gata4* gene expression (data not shown). Additionally, no correlation was found between *Asbt* and *Shp* (data not shown). Together, these findings indicate that improvement of BA malabsorption in CF mice by PEG treatment is associated with restored FXR-FGF15 signaling independent of *Asbt* expression.

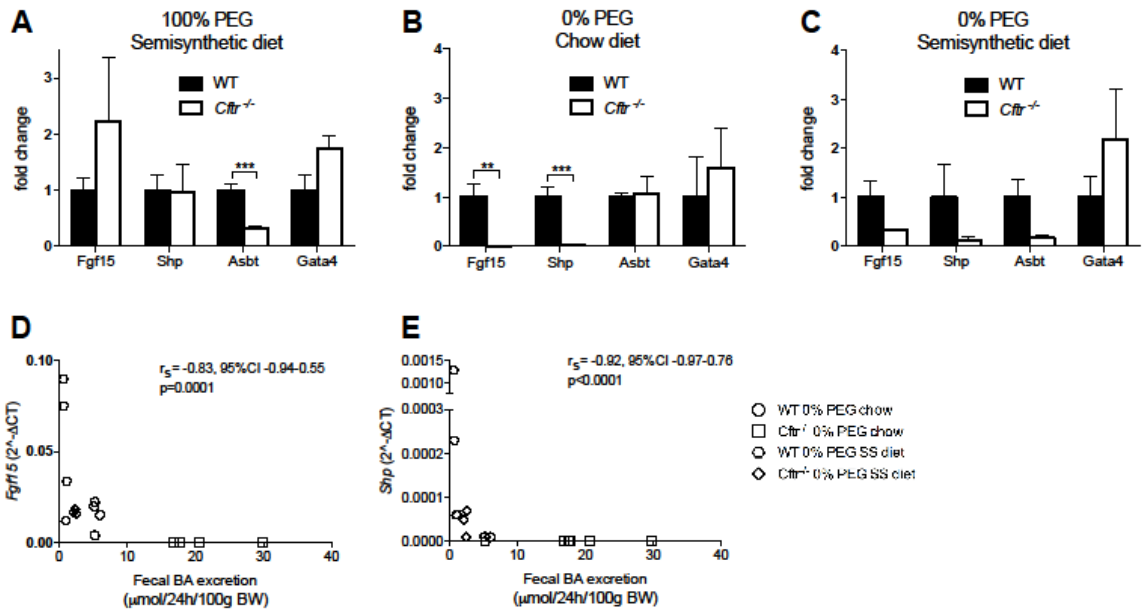


**Figure 5.** Effect of PEG on ileal gene expression in WT and CF mice (A) on 100% PEG treatment with semisynthetic diet, n=3-5 (B) on 0% PEG with chow, n=4-5 and (C) on 100% PEG with semisynthetic diet, n=6-7. Primers used are listed in Table 1. Data are

normalized to the housekeeping gene *Rplp0* (36B4) and are expressed relative to WT values. Data are shown as mean ± SE. (D) Correlation plot between fecal BA excretion and *Fgf15* and (E) Correlation plot between fecal BA excretion and *Shp*. For correlation analyses, Spearman's rank correlation coefficient was used. PEG, polyethylene glycol; *Fgf15*, fibroblast growth-factor 15; *Shp,* small heterodimer partner; *Asbt*, apical sodium-dependent bile acid transporter; *Gata4*, GATA-binding factor 4.

## DISCUSSION

In this study we show that PEG treatment completely prevented BA malabsorption in CF mice fed a semisynthetic diet, whereas this was partially prevented on a chow diet. In concomitance with improved BA absorption, FXR-FGF15 signaling was restored in CF mice fed a semi-synthetic diet by PEG treatment.

There are several mechanisms that can explain the decrease in fecal BA loss by PEG treatment. In CF, mucins remain abnormally aggregated, adhere strongly and accumulate on the epithelium (30). Such a thickened mucus layer could impair BA reabsorption by acting as a poorly penetrable barrier. PEG has previously been shown to reduce mucus accumulation in the intestine of CF mice (22) and could have therefore facilitated BA reabsorption in our study. Decreased intestinal transit time was proposed as underlying mechanism (22). We, however, did not assess the effect of PEG on mucus accumulation in intestinal crypts in the current study.

Decreased ASBT-mediated BA reuptake in CF could also be responsible for BA malabsorption. This, however, was not supported by our data. Previous studies have shown changes in *Asbt* expression in CF mouse models, either decreased or increased expression (2, 8, 20). In the current study, expression tended to be lower in CF mice upon semisynthetic diet and was unchanged upon a chow diet, suggesting that dietary factors may influence *Asbt* expression. Intestinal FXR activation has been shown to inhibit *Asbt* expression via *Shp* (23). However, here, as well as in a previous study (8), *Asbt* expression in CF mice tended to be reduced concomitantly with reduced *Shp*, suggesting that the regulation of *Asbt* expression by FXR-SHP may not be pivotal in CF. *Asbt* expression is also affected by gut microbiota, which represses expression via the transcription factor *Gata4* (26). We found no correlation between *Asbt* and *Gata4* expression. These findings suggest that other factors besides FXR and GATA4 regulate *Asbt* expression in CF. Whereas PEG treatment decreased fecal BA loss and restored

FXR-FGF15 signaling in CF mice, the ileal expression of *Asbt* was still decreased upon PEG treatment, indicating that the effects of PEG on BA homeostasis were not mediated by changes in *Asbt* expression. We cannot exclude, however, that ASBT protein function is compromised in CF and partially restored by PEG.

Impaired FXR-FGF15 signaling in untreated CF mice is reflected in the fecal BA composition, where an increased contribution of CA observed by us and others (4, 33, 36) reflects increased hepatic BA synthesis, likely due to lack of inhibition by FGF15 signaling. PEG treatment was associated with restoration of FXR-FGF15 signaling in CF mice. Our finding that PEG reduced the contribution of CA to the fecal BA pool in CF mice could reflect the increased FXR-FGF15 signaling observed upon PEG treatment. The strong correlation between fecal BA excretion and *Fgf15* and *Shp* expression suggests that FXR-FGF15 signaling was restored by improved BA reabsorption.

PEG could also have affected FXR-FGF15 signaling in CF by affecting the gut microbial composition (37). Microbiota-induced changes in the BA pool composition can modulate FXR stimulation, as microbiota-dependent BAs such as the secondary BA deoxycholic acid (DCA) are FXR agonists (31). Small intestinal bacterial overgrowth (SIBO) has been reported in CF mice fed a liquid diet (22), therefore increased BA deconjugation could be expected. Since ASBT preferentially transports conjugated rather than deconjugated BAs (13), greater fecal BA loss could be expected in CF mice with SIBO. PEG was shown to decrease SIBO in CF mice (22) and to decrease secondary BAs such as DCA in WT rats (37). Although in previous studies DCA was found in small amounts in the feces of WT and CF mice (4, 5), we could not detect any DCA or coprostanol (both microbial metabolites) upon semisynthetic diet, suggesting that the catabolic activity of the gut microbiota was decreased. This could be due to the fact that, although the semisynthetic diet contains cellulose, refined cellulose is digested poorly by the microbiota compared to cellulose

derived from dietary fiber, at least in humans (32). Furthermore, no correlation between fecal CDCA levels and Fgf15 gene expression was found, suggesting that the changes in FXR activation were not due to increased activation by CDCA. Together, these findings suggest that restoration of FXR-FGF15 signaling in CF mice occurred as a consequence of improved BA reabsorption upon PEG treatment, rather than microbiota-dependent changes in the BA composition that could have heightened FXR stimulation.

In line with previous observations (14), we found that fecal BA excretion in both genotypes was up to 10-fold higher in mice receiving chow as compared to a semisynthetic diet. The macronutrient composition, including fat, was similar across the two diets used, although more simple rather than complex carbohydrates were found in the semisynthetic diet. The fiber content and composition, however, differed greatly. By proximate analysis, the semisynthetic diet contained 10.5% of fiber, consisting exclusively of cellulose. Chow contained 4.2% of fiber, composed of cellulose (25%), hemicellulose (57%), pectin (9%) and lignin (9%). *In vitro* binding of BAs by dietary fiber has been demonstrated. Cellulose, the sole fiber in the semisynthetic diet, does not bind BAs, whereas other fibers such as pectin and lignin do, to varying extents (35). Therefore, the higher fecal BA excretion observed in chow-fed mice could be due to the presence of BA-binding fibers such as pectin and lignin in chow. Whereas we found an up to 10-fold increase in fecal BA excretion upon chow compared to semisynthetic diet, other studies reported 2-to-5-fold increases in fecal labelled cholate excretion upon chow compared to semisynthetic diet (14, 29). Besides the lack of BA-binding fiber, another mechanism that could contribute to the decreased fecal BA excretion upon semisynthetic diet compared to chow is a decrease in the microbial catabolic activity in the intestine upon feeding a semisynthetic diet. Our data show that upon semisynthetic diet there was a decrease in

coprostanol and complete lack of the secondary bile acid deoxycholic acid, suggesting that the microbial catabolic activity was decreased.

Compared to semisynthetic diet, besides increased fecal loss of BAs upon chow, we also observed increased loss of fecal NS upon chow. This could be due to the higher cholesterol content in chow (0.05%) compared to semisynthetic diet (0.01%), to decreased cholesterol absorption upon chow due to increased fecal BA loss, or to binding of cholesterol by dietary fiber along with BAs. As for binding of BAs, binding of cholesterol by cellulose was reported as negligible (15). The strong correlation between fecal BA and NS excretion could reflect all mechanisms. However, since in CF mice PEG treatment did not affect fecal NS to the extent it affected fecal BA excretion, this suggest that the effect of cholesterol binding by dietary fiber and difference in cholesterol content in the diet contributes more to this correlation.

Our study shows that, in CF mice, the osmotic laxative PEG is associated with decreased BA malabsorption and restoration of FXR-FGF15 signaling, independently from *Asbt* expression. PEG is the most commonly prescribed and most effective osmotic laxative for constipation (1) and, as constipation is common in CF and its incidence increases with age (9), CF patients are already frequently prescribed PEG. PEG is virtually free of important side effects at standard dosage (27). Besides its indication for constipation in CF, based on the evidence provided in CF mice so far, PEG could also be useful for reducing SIBO and the consequences of gut dysbiosis and inflammation in CF (22). Our study shows that FXR-FGF15 signaling can be restored by PEG in CF. Given the metabolic implications of FXR-FGF19/15 signaling, it remains to be established whether this could improve CF-related complications such as cystic fibrosis-related diabetes (CFRD) and cystic fibrosis-related liver disease (CFLD).

## Acknowledgements

We thank R. Boverhof for skillful technical assistance.

## Funding

This work was supported by the Dutch Cystic Fibrosis Society (COS17) and the De Cock Stichting. J.W.J. is further supported by the Netherlands Organization for Scientific Research (VIDI grant 016.126.338).

## REFERENCES


1. **Belsey JD**, **Geraint M**, **Dixon TA**. Systematic review and meta analysis: polyethylene glycol in adults with non-organic constipation. *Int J Clin Pract* 64: 944–955, 2010.
2. **Benten D**, **Wiesch JS zur**, **Sydow K**, **Koops A**, **Buggisch P**, **Böger RH**, **Gaydos CA**, **Won H**, **Franco V**, **Lohse AW**, **Ray SC**, **Balagopal A**. The transhepatic endotoxin gradient is present despite liver cirrhosis and is attenuated after transjugular portosystemic shunt (TIPS). *BMC Gastroenterol* 11: 107, 2011.
3. **Bijvelds MJC**, **Bronsveld I**, **Havinga R**, **Sinaasappel M**, **de Jonge HR**, **Verkade HJ**. Fat absorption in cystic fibrosis mice is impeded by defective lipolysis and post-lipolytic events. *Am J Physiol Gastrointest Liver Physiol* 288: G646-53, 2005.
4. **Bodewes FAJA**, **van der Wulp MYM**, **Beharry S**, **Doktorova M**, **Havinga R**, **Boverhof R**, **James Phillips M**, **Durie PRR**, **Verkade HJJ**. Altered intestinal bile salt biotransformation in a cystic fibrosis (Cftr-/-) mouse model with hepato-biliary pathology. *J Cyst Fibros* 14: 440–446, 2015.
5. **Bodewes FAJAJA**, **Bijvelds MJ**, **De Vries W**, **Baller JFWW**, **Gouw ASHH**, **De Jonge HR**, **Verkade HJ**. Cholic acid induces a Cftr dependent biliary secretion and liver growth response in mice. *PLoS One* 10: 1–14, 2015.
6. **Borowitz D**, **Durie PR**, **Clarke LL**, **Werlin SL**, **Taylor CJ**, **Semler J**, **De Lisle RC**, **Lewindon P**, **Lichtman SM**, **Sinaasappel M**, **Baker RD**, **Baker SS**, **Verkade HJ**, **Lowe ME**, **Stallings VA**, **Janghorbani M**, **Butler R**, **Heubi J**. Gastrointestinal outcomes and confounders in cystic fibrosis. *J Pediatr Gastroenterol Nutr* 41: 273–285, 2005.
7. **Clarke LL**, **Gawenis LR**, **Franklin CL**, **Harline MC**. Increased Survival of CFTR Knockout Mice with an Oral Osmotic Laxative. *Lab Anim Sci* 46: 612–618, 1996.
8. **Debray D**, **Rainteau D**, **Barbu V**, **Rouahi M**, **El Mourabit H**, **Lerondel S**, **Rey C**, **Humbert L**, **Wendum D**, **Cottart C**, **Dawson P**, **Chignard N**, **Housset C**. Defects in Gallbladder Emptying and Bile Acid Homeostasis in Mice With Cystic Fibrosis Transmembrane Conductance Regulator Deficiencies. *Gastroenterology* 142: 1581–1591.e6, 2012.
9. **van der Doef HPJ**, **Kokke FTM**, **Beek FJA**, **Woestenenk JW**, **Froeling SP**, **Houwen RHJ**.

Constipation in pediatric Cystic Fibrosis patients: An underestimated medical condition. *J Cyst Fibros* 9: 59–63, 2010.

10. **Fang Q**, **Li H**, **Song Q**, **Yang W**, **Hou X**, **Ma X**, **Lu J**, **Xu A**, **Jia W**. Serum Fibroblast Growth Factor 19 Levels Are Decreased in Chinese Subjects With Impaired Fasting Glucose and Inversely Associated With Fasting Plasma Glucose Levels. *Diabetes Care* 36: 2810–2814, 2013.

11. **Freudenberg F**, **Broderick AL**, **Yu BB**, **Leonard MR**, **Glickman JN**, **Carey MC**. Pathophysiological basis of liver disease in cystic fibrosis employing a DeltaF508 mouse model. *Am J Physiol Gastrointest Liver Physiol* 294: G1411-20, 2008.

12. **Garg M**, **Ooi CY**. The Enigmatic Gut in Cystic Fibrosis: Linking Inflammation, Dysbiosis, and the Increased Risk of Malignancy. *Curr Gastroenterol Rep* 19: 6, 2017.

13. **Geyer J**, **Wilke T**, **Petzinger E**. The solute carrier family SLC10: more than a family of bile acid transporters regarding function and phylogenetic relationships. *Naunyn Schmiedebergs Arch Pharmacol* 372: 413–431, 2006.

14. **Gustafsson BE**, **Norman A**. Influence of the diet on the turnover of bile acids in germ-free and conventional rats. *Br J Nutr* 23: 429–442, 1969.

15. **Hu G**, **Yu W**. Binding of cholesterol and bile acid to hemicelluloses from rice bran. *Int J Food Sci Nutr* 64: 461–466, 2013.

16. **Kelly A**, **Moran A**. Update on cystic fibrosis-related diabetes. *J Cyst Fibros* 12: 318–331, 2013.

17. **Kir S**, **Beddow SA**, **Samuel VT**, **Miller P**, **Previs SF**, **Suino-Powell K**, **Xu HE**, **Shulman GI**, **Kliewer SA**, **Mangelsdorf DJ**. FGF19 as a Postprandial, Insulin-Independent Activator of Hepatic Protein and Glycogen Synthesis. *Science (80- )* 331: 1621–1624, 2011.

18. **Kuipers F**, **Bloks VW**, **Groen AK**. Beyond intestinal soap - bile acids in metabolic control. *Nat Rev Endocrinol* 10: 488–498, 2014.

19. **Li L**, **Somerset S**. The clinical significance of the gut microbiota in cystic fibrosis and the potential for dietary therapies. *Clin Nutr* 33: 571–580, 2014.

20. **De Lisle RC**. Decreased Expression of Enterocyte Nutrient-Assimilation Genes and

Proteins in the Cystic Fibrosis Mouse Small Intestine. *J Pediatr Gastroenterol Nutr* 62: 1, 2015.

21. **De Lisle RC**, **Borowitz D**. The cystic fibrosis intestine. *Cold Spring Harb Perspect Med* 3: 1–17, 2013.

22. **De Lisle RC**, **Roach E**, **Jansson K**. Effects of laxative and N-acetylcysteine on mucus accumulation, bacterial load, transit, and inflammation in the cystic fibrosis mouse small intestine. *Am J Physiol Gastrointest Liver Physiol* 293: G577-84, 2007.

23. **Neimark E**, **Chen F**, **Li X**, **Shneider BL**. Bile acid-induced negative feedback regulation of the human ileal bile acid transporter. *Hepatology* 40: 149–156, 2004.

24. **O'Brien S**, **Mulcahy H**, **Fenlon H**, **O'Broin A**, **Casey M**, **Burke A**, **FitzGerald MX**, **Hegarty JE**. Intestinal bile acid malabsorption in cystic fibrosis. *Gut* 34: 1137–1141, 1993.

25. **O'Sullivan BP**, **Freedman SD**. Cystic fibrosis. *Lancet* 373: 1891–1904, 2009.

26. **Out C**, **Patankar J V.**, **Doktorova M**, **Boesjes M**, **Bos T**, **De Boer S**, **Havinga R**, **Wolters H**, **Boverhof R**, **Van Dijk TH**, **Smoczek A**, **Bleich A**, **Sachdev V**, **Kratky D**, **Kuipers F**, **Verkade HJ**, **Groen AK**. Gut microbiota inhibit Asbt-dependent intestinal bile acid reabsorption via Gata4. *J Hepatol* 63: 697–704, 2015.

27. **Pashankar DS**, **Loening-Baucke V**, **Bishop WP**. Safety of polyethylene glycol 3350 for the treatment of chronic constipation in children. *Arch Pediatr Adolesc Med* 157: 661–664, 2003.

28. **Van De Peppel IP**, **Doktorova M**, **Berkers G**, **de Jonge HR**, **Houwen RHJJ**, **Verkade HJ**, **Jonker JW**, **Bodewes FAJAJA**. IVACAFTOR restores FGF19 regulated bile acid homeostasis in cystic fibrosis patients with an S1251N or a G551D gating mutation. *J Cyst Fibros* 50: 297, 2018.

29. **Portman OW**, **Murphy P**. Excretion of bile acids and β-hydroxysterols by rats. *Arch Biochem Biophys* 76: 367–376, 1958.

30. **Quinton PM**. Cystic fibrosis: impaired bicarbonate secretion and mucoviscidosis. *Lancet* 372: 415–417, 2008.

31. **Ridlon JJM**, **Kang D-JD**, **Hylemon PBP**, **Bajaj JJS**. Bile Acids and the Gut Microbiome. *Curr Opin Gastroenterol* 30: 332–338, 2014.

32. **Ronda OAHO**, **van Dijk TH**, **Verkade HJ**, **Groen AK**. Measurement of Intestinal and Peripheral Cholesterol Fluxes by a Dual-Tracer Balance Method. *Curr Protoc Mouse Biol* 6: 408–434, 2016.

33. **Strandvik B**, **Einarsson K**, **Lindblad A**, **Angelin B**. Bile acid kinetics and biliary lipid composition in cystic fibrosis. *J Hepatol* 25: 43–48, 1996.

34. **Tremaroli V**, **Bäckhed F**. Functional interactions between the gut microbiota and host metabolism. *Nature* 489: 242–249, 2012.

35. **Vahouny G V**. Dietary Fiber, Bile Acids and Cholesterol Metabolism. In: *Nutritional Biochemistry and Pathology*, edited by Santos W, Lopes N, Barbosa JJ, Chaves D, Valente JC. Boston, MA: Springer US, 1980, p. 473–485.

36. **Wouthuyzen-Bakker M**, **Bijvelds MJ**, **de Jonge HR**, **De Lisle RC**, **Burgerhof JG**, **Verkade HJ**. Effect of antibiotic treatment on fat absorption in mice with cystic fibrosis. *Pediatr Res* 71: 4–12, 2012.

37. **van der Wulp MYM**, **Derrien M**, **Stellaard F**, **Wolters H**, **Kleerebezem M**, **Dekker J**, **Rings EHHM**, **Groen AK**, **Verkade HJ**. Laxative treatment with polyethylene glycol decreases microbial primary bile salt dehydroxylation and lipid metabolism in the intestine of rats. *Am J Physiol Gastrointest Liver Physiol* 305: G474-82, 2013.

38. **Zhou M**, **Learned RM**, **Rossi SJ**, **Depaoli AM**, **Tian H**, **Ling L**. Engineered fibroblast growth factor 19 reduces liver injury and resolves sclerosing cholangitis in Mdr2-deficient mice. *Hepatology* 63: 914–929, 2016.

39. **Zhou M**, **Learned RM**, **Rossi SJ**, **DePaoli AM**, **Tian H**, **Ling L**. Engineered FGF19 eliminates bile acid toxicity and lipotoxicity leading to resolution of steatohepatitis and fibrosis in mice. *Hepatol Commun* 1: 1024–1042, 2017.